REMARKS

Reconsideration is respectfully requested in view of the above amendments and following remarks. Claim 27 is amended and supported in Applicants' experimental results. No new matter has been added. Claims 15, 16, and 27 are pending.

Rejections under 35 U.S.C. 112

Claims 15, 16, and 27 are rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. Applicants respectfully traverse this rejection to the extent it is maintained.

The rejection alleges that the preparations of the compounds recited in claim 27 are not set forth to enable one skilled in the art to make and use the invention. Applicants respectfully disagree and submit that the claims are enabled because the compounds were known at the time of filing the present application. For example, Applicants respectfully submit that US 5,972,976 (corresponding US patent of PCT publication WO 95/27699 and attached herewith) teaches the availability and synthesis of the compounds claimed. See e.g. Column 3, line 64 through Column 4, line 67. For at least these reasons, claims 15, 16, and 27 are enabled.

Favorable reconsideration and withdrawal of the rejection are respectfully requested.

Claims 15, 16, and 27 also are rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. Applicants respectfully traverse this rejection to the extent it is maintained.

Claim 27 has been amended to include the language "synergistic inhibitory effect" as suggested by the Examiner. Applicants appreciate the Examiner's suggestion, and request withdrawal of the rejection.

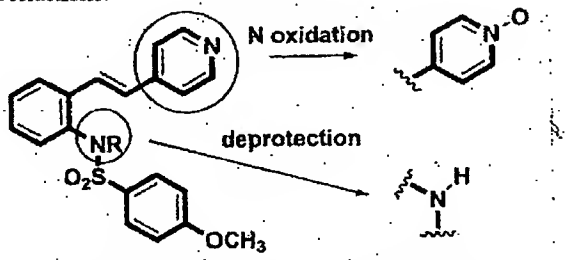
Rejection under 35 U.S.C. 103(a)

Claims 15, 16, and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over reference combinations including Hidaka et al. (US 5972976) in view of Goodman and Gilman, *The Pharmacological Basis of therapeutics* and Ragaz et al., *The New*

England J. of Med. Applicants respectfully traverse the rejection to the extent it is maintained.

The rejection has been maintained because it is alleged that unexpected results are only shown with respect to the combination of the second compound (E)-4-[2-[2-[N-acetyl-N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine 1-oxide and cisplatin, but are not shown in combination with carboplatin, or in combinations of the other compounds claimed with cisplatin or carboplatin. Applicants respectfully disagree and submit that one of skill in the art would expect that the other claimed compounds would exhibit similar synergistic effects when combined with cisplatin, because the compounds possess structural and reactive similarities. Further, one of skill in the art would expect similar synergistic effects when any of the compounds claimed are combined with carboplatin, because carboplatin is an analog of cisplatin with essentially the same reactive structure.

With reference to the compounds, not only the second compound (E)-4-[2-[2-[N-acetyl-N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine 1-oxide, but also the other claimed compounds would reasonably have the expectation of showing synergistic effects. When metabolized in a living body, the claimed six compounds are well known to convert the pyridine moiety to a pyridine N-oxide group through oxidation and are well known to undergo deprotection of an NR group to convert it to an NH group. See the following mechanism:



Therefore, one of skill in the art would recognize that not only the second compound but also the four other claimed compounds (E)-4-[2-[2-[N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine, (E)-4-[2-[2-[N-acetyl-N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine, (E)-4-[2-[2-[N-(2-hydroxyethyl)-N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine 1-oxide,

and (E)-4-[2-[2-[N-(2-hydroxyethyl)-N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine, would be converted to the third compound (E)-4-[2-[2-[N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine 1-oxide. This is further supported, for example in the graph (see Appendix submitted herewith) which shows the disposition of the first compound (E)-4-[2-[2-[N-[(p-methoxyphenyl)sulfonyl]amino]phenyl]ethenyl]pyridine. The graph clearly shows that the first compound is converted to the third compound *in vivo*. Furthermore, the third compound is well known as one main active form of the group of compounds claimed and is known to exhibit anti-tumor activity. See e.g. compound of Example 1 in US 5,972,976. Therefore, it is clear that one of skill in the art would expect that the claimed compounds of claim 27 are converted to the third compound and would expect the compounds to show anti-tumor activity. The specification shows the effects for the second compound, and it is neither necessary, nor reasonable to require examples showing the effects of all the compounds claimed given their structural and reactive similarities.

Regarding combinations of the claimed compounds with the anticancer agent carboplatin, one of skill in the art would expect similar synergistic effects from such combinations, because carboplatin exerts a similar effect as cisplatin in a living body. This is supported, for example in *CANCER, Principles & Practice of Oncology*, 7th Edition, p.344-358 (2005), which is submitted herewith. See e.g. page 345, Platinum Chemistry and page 347, Mechanism of Action. Even further, carboplatin and cisplatin are common diamino compounds of platinum (II), where carboplatin forms an adduct with a DNA that has essentially the same structure as that formed in a reaction between cisplatin and a DNA. See e.g. arrow indication at the top of page 348. Thus, one of skill in the art would expect carboplatin to behave similarly as cisplatin. For at least the foregoing reasons, synergistic inhibitory effect at such supported dosages would be expected from any of the compounds claimed when combined with either cisplatin or carboplatin.

Turning to the references cited, the references cited do not teach or suggest claim 27, namely any of the six compounds claimed in combination with the recited

other antitumor agent. For at least this reason, claim 27 and its dependent claims 15 and 16 are patentable.

Moreover, the claimed invention provides unexpected advantageous results in that an antitumor effect can be increased while toxicity of respective agents can be reduced. That is, the present invention can provide enhanced therapeutic effect while decreasing side effects. As discussed above, the rejection contends that the disclosure relied upon is insufficient to show unexpected results for all combinations of compounds with either cisplatin or carboplatin. Applicants respectfully disagree and contend that, based on at least the evidence of record and the foregoing reasons, unexpected results for synergistic anti-tumor effects are shown for the scope of the claimed invention. Thus, the claimed invention is not obvious over Hidaka et al, Goodman, and Ragaz. For at least the foregoing reasons, claim 15-16, and 27 are patentable.

Favorable reconsideration and withdrawal of the rejection are respectfully requested.

A Notice of Allowance is respectfully solicited. Any questions or concerns regarding this communication can be directed to Applicants' representative listed below.

Respectfully submitted,

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Dated: January 19, 2009

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Reg. No. 30,300

Appendix

Disposition of HMN compounds

1. Generation of HMN-176 from HMN-154

1.1 Generation of HMN-176 in vivo

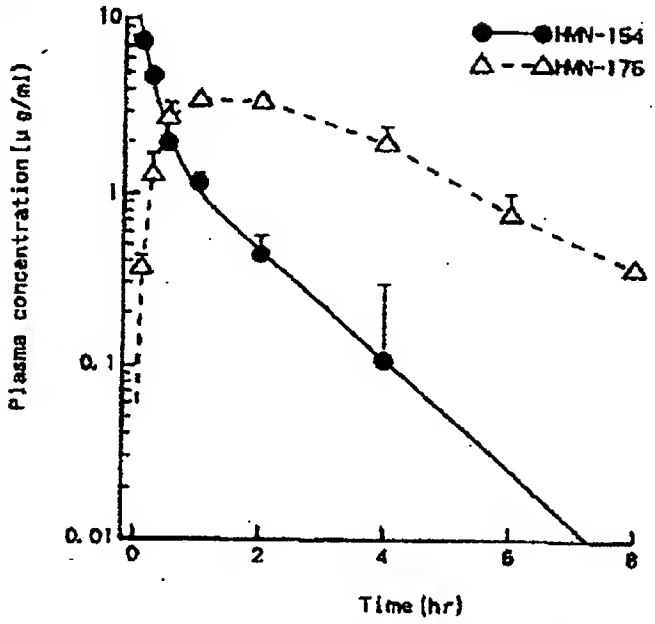

Figure 1
Transition of concentrations of HMN-154 and HMN-176 in plasma after intravenous administration of 10 mg/kg HMN-154.
Solid line is a theoretical curve.

HMN-154: The first compound in claim 27

HMN-176: The third compound in claim 27

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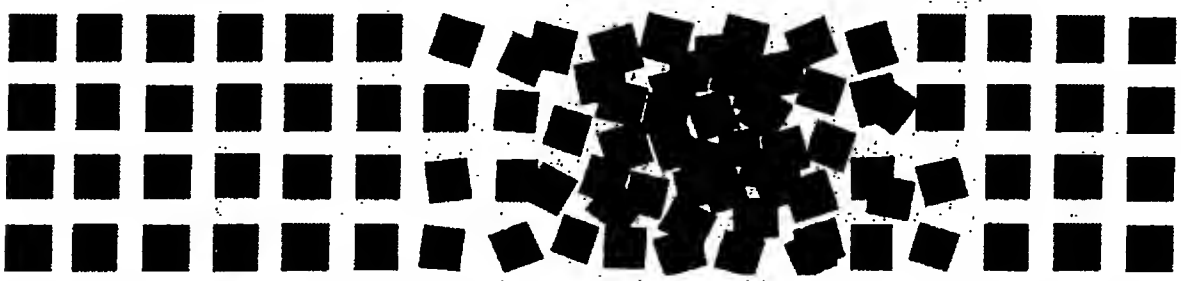

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Vernon K. Sondak, MD, serves on the Speakers Bureau of Schering Oncology Biotech.

Ronald M. Summers, MD, PhD, has patents in the subject area of his chapter.

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SECTION **5**

STEVEN W. JOHNSON
PETER J. O'DWYER

Cisplatin and Its Analogues

The platinum drugs represent a unique and important class of antitumor compounds. Alone or in combination with other chemotherapeutic agents, *cis*-diamminedichloroplatinum (II) (cisplatin) and its analogues have made a significant impact on the treatment of a variety of solid tumors for nearly 30 years. The unique activity and toxicity profile observed with cisplatin in early clinical trials fueled the development of platinum analogues that are less toxic and more active against a variety of tumor types, including those that have developed resistance to cisplatin. In addition to cisplatin, two other platinum complexes are currently approved for use in the United States: *cis*-diamminecyclobutanedicarboxylato platinum (II) (carbo 1,2-diaminocyclohexaneoxalato platinum (II) (oxal addition to these, several other analogues with uniq are in various stages of clinical development. progress in the development of superior analogues thorough understanding of the chemical, biologic, p netic, and pharmacodynamic properties of this imp of drugs. A review of these properties is the focus of t

HISTORY

The realization that platinum complexes exhibitec activity began somewhat serendipitously in a serie ments carried out by Dr. Barnett Rosenberg and beginning in 1961.[1] These studies involved deter effect of electromagnetic radiation on the growth o a chamber equipped with a set of platinum electr sure of the bacteria to an electric field resulted in

change in their morphology, in particular, the appearance of long filaments that were several hundred times longer than that of their unexposed counterparts. This effect was not due directly to the electric field, but to the electrolysis products produced by the platinum electrodes. An analysis of these products revealed that the predominant species was ammonium chloroplatinate $[NH_4]_2[PtCl_6]$. This compound was inactive in reproducing the filamentous growth originally observed; however, Rosenberg and colleagues soon discovered that the conversion of this complex to a neutral species by ultraviolet light resulted in an active species. Attempts to synthesize the active neutral platinum complex failed. They realized, however, that the neutral compound could exist in two isomeric forms, *cis* or *trans*, and the latter species is the one that they had synthesized. Subsequently, the *cis* isomer was synthesized and shown to be the active compound.

The observation that *cis*-diamminedichloroplatinum (II) and *cis*-diammine tetrachloroplatinum (IV) inhibited bacterial growth led to the testing of four neutral platinum compounds for antineoplastic activity in mice bearing the Sarcoma-180 solid tumor and L1210 leukemia cells.[2] All four compounds showed significant antitumor activity, with *cis*-diamminedichloroplatinum (II) exhibiting the most efficacy. Further studies in other tumor models confirmed these results and indicated that cisplatin exhibited a broad spectrum of activity. Although early clinical trials demonstrated significant activity against several tumor types, particularly testicular cancers, the severe renal and gastrointestinal toxicity caused by the drug nearly led to its abandonment. Cvitkovic and colleagues[3,4] showed that these effects could be ameliorated, in part, by aggressive prehydration, which rekindled interest in its clinical use. Currently, cisplatin is curative in testicular cancer and significantly prolongs survival in combination regimens for ovarian cancer. The drug also has therapeutic benefit in head and neck, bladder, and lung cancer.[5] Continued study is demonstrating activity in other tumors as well.

PLATINUM CHEMISTRY

Platinum exists primarily in either a 2+ or 4+ oxidation state. These oxidation states dictate the stereochemistry of the carrier ligands and leaving groups surrounding the platinum atom. Platinum (II) compounds exhibit a square planar geometry, whereas platinum (IV) compounds exhibit an octahedral geometry. Interconversion of the two oxidation states may readily occur; however, the kinetics of this reaction depend on the nature of the bound ligands. The nature of the ligands also determines the stability of the complex and the rate of substitution. For platinum (II) compounds, the rate of substitution of a ligand is strongly influenced by the type of ligand located opposite to it. Therefore, ligands that are bound more strongly will stabilize the moieties that are situated *trans* to it. For *cis*-diamminedichloroplatinum (II), the two chloride ligands are prone to substitution, whereas substitution of the amino groups is thermodynamically unfavorable.[6] The stereochemistry of platinum complexes is critical to their antitumor activity as evidenced by the significantly reduced efficacy observed with *trans*-diamminedichloroplatinum (II).

In aqueous solution, the chloride leaving groups of cisplatin are subject to mono- and diaqua substitution, particularly at chloride concentrations below 100 mmol, which exist intracellularly. The equilibria may be described by the following two equations:

$$cis\text{-}(NH_3)_2PtCl_2 + H_2O \leftrightarrows Cl^- + cis\text{-}(NH_3)_2PtCl(H_2O)^+$$

$$cis\text{-}(NH_3)_2PtCl(H_2O)^+ + H_2O \leftrightarrows Cl^- + cis\text{-}(NH_3)_2Pt(H_2O)_2^{2+}$$

where equilibrium constants for each reaction may be written:

$$K_1 = \frac{[Cl^-][cis\text{-}(NH_3)_2PtCl(H_2O)^+]}{[cis\text{-}(NH_3)_2PtCl_2]} \text{ and.}$$

$$K_2 = \frac{[Cl^-][cis\text{-}(NH_3)_2Pt(H_2O)2^{2+}]}{[cis\text{-}(NH_3)_2PtCl(H_2O)^+]}$$

These descriptions illustrate the key role of ambient chloride concentrations in determining aquation rates. In weakly acidic solutions, the monochloromonoaqua and diaqua complexes become deprotonated to form the neutral dihydroxo species. The monohydroxo and dihydroxo complexes are the predominant species present in low chloride-containing environments such as the nucleus. A detailed analysis of the equations and rate constants that govern these reactions has n been published.[7] Based on studies of the reaction of cisplatin metabolites with inosine, the predominant cisplatin species that react with DNA are likely to be the chloroaqua and hydroxoaqua species.[7]

NOVEL PLATINUM COMPLEXES

Early in the clinical development of cisplatin it became clear that its toxicity was a barrier to widespread acceptance and that its activity, although striking in certain diseases, did not extend to all cancers. These observations simultaneously gave rise to approaches to modifying toxicity and to the search for structural analogues with activity in cisplatin-resistant tumor models. In addition to stimulation of the development of antiemetics and other supportive care measures for use with cisplatin, structural modifications in the molecule were sought to alter the tissue distribution. Progress in understanding the chemistry and pharmacokinetics of cisplatin has guided the development of new analogues. In general, modification of the chloride leaving groups of cisplatin results in compounds with different pharmacokinetics, whereas modification of the carrier ligands alters the activity of the resulting complex. This section summarizes the features of the more important platinum analogues that have been developed, which are shown in Figure 15.5-1.

CARBOPLATIN

Substitution of the chloride leaving groups of cisplatin resulted in compounds with diminished nephrotoxicity but equivalent activity. Using a murine screen for nephrotoxicity, it was discovered that substituting a cyclobutanedicarboxylate moiety for the two chloride ligands of cisplatin resulted in a complex with reduced renal toxicity. This observation was translated to the clinic in the form of carboplatin, a more stable and pharmacokinetically predictable analogue.[8,9] The results in humans were accurately predicted by the animal models, and marrow toxicity rather than nephrotoxicity was the principal side effect. At effective doses,

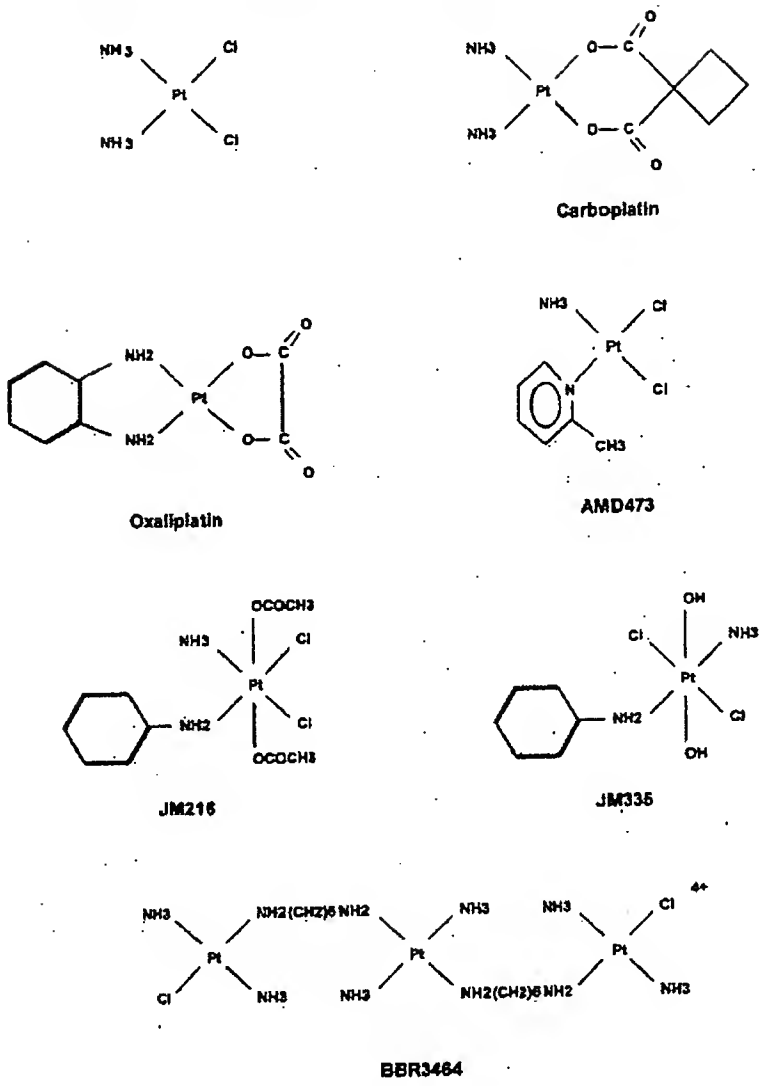

FIGURE 15.5-1. Structures of cisplat analogues.

carboplatin produced less nausea, vomiting, nephrotoxicity, and neurotoxicity than cisplatin. Furthermore, the myelosuppression was closely associated with the pharmacokinetics. The work of Calvert et al.[10] and Egorin and colleagues[11] showed that toxicity can be made more predictable and dose intensity less variable by dosing strategies based on the exposure. Carboplatin was shown to be indistinguishable from cisplatin in its clinical activity in all but a handful of tumor types and is the most frequently used form of platinum in current use.

1,2-DIAMINOCYCLOHEXANE DERIVATIVES

Compounds with activity in cisplatin-resistant models emerged from modifications to the carrier group (left side of the analogues in Fig. 15.5-1). The pioneer in this field was Dr. Tom Connors, who in the late 1960s synthesized platinum coordination compounds with varying physicochemical characteristics and found that the series that possessed a diaminocyclohexane (DACH rier group was active in cell culture models of cancer.[12] Bur et al. provided *in vivo* confirmation that these structure indeed active in solid tumors and leukemias in which cispla little or no activity.[15] Subsequent *in vitro* studies supported th that DACH-based platinum complexes were non–cross-resis cisplatin-resistant cell lines.[14,15] In support of these studies, al.[16] showed that DACH derivatives exhibited a unique cyto profile compared to cisplatin and carboplatin using the N Cancer Institute 60 cell line screen.

An early analogue that was developed out of this work wa platin (ormaplatin), which underwent a relatively slow d ment over the next 20 years, culminating in phase I trials early 1990s. The severe neurotoxicity of the agent led to it donment. Attention had already focused, however, on a DACH analogue that had been synthesized by Kidani a leagues in the early 1970s and had undergone a simila

gestation into the clinic. Oxaliplatin, a coordination compound of a DACH carrier group and an oxalato leaving group, is substantially less lipophilic than tetraplatin but retains the latter's spectrum of activity in cisplatin-resistant tumor models. Like cisplatin, oxaliplatin preferentially forms adducts at the N7 position of guanine and to a lesser extent adenine. However, there is evidence that the three-dimensional structure of the DNA adducts and biologic response(s) they elicit are different from those of cisplatin. Oxaliplatin was first studied in two phase I trials in which suitable doses and schedules were determined, and an early hint of colorectal cancer activity was identified.[17,18] Oxaliplatin demonstrated activity in combination with 5-fluorouracil and leucovorin in colon cancer, a disease that was previously considered to be unresponsive to platinum drugs.[19] There followed a series of consistent phase II and III clinical trial results showing the activity of oxaliplatin in colorectal cancer. Oxaliplatin is now approved for the first-line treatment of advanced colorectal cancer, and preliminary data indicate that it improves the survival of patients with stage II and III disease when used in the adjuvant setting. The potential of oxaliplatin in other diseases is at an early stage of exploration, and additional therapeutic applications may emerge.

PLATINUM (IV) STRUCTURES

The octahedral stereochemistry adopted by platinum (IV) compounds has led investigators to speculate that they may exhibit a different spectrum of activity than that of platinum (II) drugs. Two compounds that have been tested clinically without much success are ormaplatin and iproplatin. Ormaplatin was neurotoxic in phase I trials, and iproplatin failed to demonstrate activity in phase II trials.[20–22] More recently, two platinum (IV) compounds, JM216 [bis(acetato)amminedichloro(cyclohexylamine) platinum (IV)] and JM335 [*trans*-ammine(cyclohexylamine)dichlorodihydroxo platinum (IV)], have been developed and contain several unique features.[23] These compounds may also be classified as mixed amines or ammine-amine platinum (IV) complexes. JM216 is the first orally active platinum compound; it has undergone extensive clinical testing in phase II and III trials.[24,25] Some activity has been noted in lung cancer (small cell and non–small cell) and in ovarian cancer, but more marked activity has been associated with its use in prostate cancer. A small, randomized trial involving 50 patients suggested a benefit for the combination of JM216 (now called satraplatin) and prednisone over prednisone alone in hormone-resistant disease.[26] A definitive phase III trial is under way for this indication.

Based on the lack of antitumor activity of transplatin [*trans*-diamminedichloroplatinum (II)], it has been generally believed that most, if not all, *trans* platinum compounds were inactive. Renewed interest in *trans* compounds has occurred, however, with the observation that JM335 and a related group of complexes exhibited significant antitumor activity in murine ADJ/PC6 and human ovarian cancer models.[23] Siddik and colleagues[27] have also produced *trans* platinum (IV) compounds containing the DACH moiety, which they demonstrated to be non–cross-resistant to cisplatin.

MULTINUCLEAR PLATINUM COMPLEXES

An approach based on the chemistry of the platinum-DNA interaction led to design and synthesis by Farrell et al.[28] of a novel class of compounds containing multiple platinum atoms (see Fig. 15.5-1). These bi- and trinuclear structures form adducts that span greater distances across the minor groove of DNA and have a profile of cell kill that differs from that of the small molecules. These compounds are unique in that their interaction with DNA is considerably different from that of cisplatin, particularly in the abundance of interstrand cross-links formed. Also, the observation that multinuclear platinum complexes containing the *trans* geometry exhibit antitumor activity contradicts the original dogma that platinum drugs containing the *trans* geometry are inactive. Currently, the lead compound in this class of drugs is BBR3464. Its structure is described as two *trans*-$[PtCl(NH_3)_2]^+$ units linked together by a noncovalent tetraamine $[Pt(NH_3)_2[H_2N(CH_2)_6NH_2]_2]^{2+}$ unit. Preclinical testing of BBR3464 shows it to be significantly more potent than cisplatin and to be active in cisplatin-resistant xenografts and p53 mutant tumors. Information on the clinical activity of BBR3464 awaits the completion of phase II trials.

OTHER PLATINUM COMPLEXES

Efforts have been made to design novel platinum analogues that can circumvent known cisplatin resistance mechanisms. An example of this is *cis*-amminedichloro(2-methylpyridine, platinum (II) (also known as *AMD473* and *ZD0473*). This compound is a sterically hindered platinum complex that was designed to have minimal reactivity with thiols and thus avoid inactivation by molecules such as glutathione.[29,30] A number of platinum drugs are in clinical trials, and there is interest in defining a profile different from that of the currently approved agents. ZD0473 was studied in phase I and had but brief phase II trials.[31,32] Responses were identified with its use, and myelosuppression was dose limiting. Other toxicities were mild with this agent. Continuing clinical research is likely. A major goal of current research is to identify the molecular characteristics of tumors that predispose them to response to one or another of the analogues. This information can then be used to refine and individualize treatment.

MECHANISM OF ACTION

DNA ADDUCT FORMATION

The observation by Rosenberg[1] that cisplatin induces filamentous growth in bacteria without affecting RNA and protein synthesis implicated DNA as the cytotoxic target of the drug. Evidence from several subsequent experiments supported this idea.[33–37] The differential cytotoxic effects observed with platinum drugs are determined, in part, by the structure and relative amount of DNA adducts formed. Cisplatin and its analogues react preferentially at the N7 position of guanine and adenine residues to form a variety of monofunctional and bifunctional adducts.[38] The first step of the reaction involves the formation of monoadducts. These monoadducts may then react further to form intrastrand or interstrand cross-links. The predominate bidentate lesions that are formed with DNA *in vitro* or in cultured cells are the d(GpG)Pt, d(ApG)Pt, and d(GpNpG)Pt intrastrand cross-links. Cisplatin also forms interstrand cross-links between guanine residues located on opposite strands that account for fewer than 5% of the total DNA-bound platinum.

These adducts may contribute to the drug's cytotoxicity because they impede certain cellular processes that require the separation of both DNA strands, such as replication and transcription.

The adducts that are formed in the reaction between carboplatin and DNA in cultured cells are essentially the same as those of cisplatin; however, higher concentrations of carboplatin are required (20- to 40-fold for cells) to obtain equivalent total platinum-DNA adduct levels due to its slower rate of aquation.[39] As with cisplatin, a relatively low number of monoadducts and interstrand cross-links are observed. The relative amounts and frequencies of the DNA adducts formed in cultured cells by oxaliplatin has also been examined. Oxaliplatin intrastrand adducts form more slowly due to a slower rate of conversion from monoadducts; however, they are formed at similar DNA sequences and regions as cisplatin adducts. Saris et al.[40] reported that oxaliplatin forms predominantly d(GpG)Pt and d(ApG)Pt intrastrand cross-links *in vitro* and in cultured cells; however, at equitoxic doses, oxaliplatin forms fewer DNA adducts than does cisplatin. This suggests that oxaliplatin lesions are more cytotoxic than those formed by cisplatin.

The differences observed in cytotoxicity between the diammine (e.g., cisplatin, carboplatin) and DACH platinum compounds does not appear to depend on the type and relative amounts of the adducts formed but is more likely due to the overall three-dimensional structure of the adduct and its recognition by various cellular proteins. Structural analysis of the cisplatin d(GpG)Pt intrastrand cross-link has been accomplished by both x-ray crystallography and nuclear magnetic resonance spectroscopy. These studies revealed that the binding of platinum to DNA causes a variety of perturbations in the double helix, including a roll of 26 to 50 degrees between the cross-linked guanine bases, displacement of platinum from the planes of the guanine rings, a bend of the helical axis toward the major groove, and an unwinding of the DNA.[41] Scheeff et al.[42] used computer modeling to demonstrate that oxaliplatin produces a similar DNA bend, base rotation, and base propeller as cisplatin. The major difference, however, is the protrusion of the DACH moiety of oxaliplatin into the major groove of DNA, which thus produces a bulkier adduct than that of cisplatin. This bulkier, more hydrophobic adduct may be recognized differently by a host of cellular proteins involved in sensing DNA damage.[43] The functional consequences of these effects are twofold: Proteins such as polymerases that recognize and participate in reactions on DNA under normal circumstances may be perturbed, whereas processes that are controlled by proteins that recognize damaged DNA may become activated. The latter group of proteins may function in the DNA repair process or in the initiation of programmed cell death.

DAMAGE RECOGNITION, SURVIVAL, AND APOPTOSIS

The sequence of events that leads to cell death after the formation of platinum-DNA adducts has not yet been elucidated; however, cells treated with platinum drugs display the biochemical and morphologic features of apoptosis.[44] These features are common to cells treated with other cytotoxic and biologic agents. Therefore, understanding the pathway(s) that are involved in the early stages of programmed cell death, including the detection-initiation and decision-commitment phases, is important for understanding the unique activities of platinum drugs. The sensitivity of a cell to a platinum drug depe part, on cell cycle. For example, proliferating cells are re sensitive, whereas quiescent cells or cells in G_0 or G_1 a tively insensitive.[45] Thus, it is possible that programm death initiated at various cell-cycle checkpoints is gover different proteins and signal transduction pathways.

A model for cisplatin-induced cell death has been pr by Sorenson and Eastman.[46] using DNA repair-deficie nese hamster ovary (CHO) cells. In these studies, cis treated CHO/AA8 cells experienced slow progression th S phase and accumulated in G_2. At low drug concentr the cells recovered and continued to cycle. At high dru centrations, the cells died after a protracted G_2 arrest. Ar rant mitosis was observed before apoptosis. Further with G_2-synchronized cells revealed that passage thro phase is necessary for G_2 arrest and cell death, which su that DNA replication on a damaged template may result accumulation of further damage, causing the cells to ulti die. Abrogating the G_2 checkpoint with pharmacologic such as caffeine or 7-hydroxystaurosporine was sho enbance the cytotoxicity of cisplatin.[47] It is not yet clea these events specifically transduce a proapoptotic signal ever, the observations provide a valuable framework to be elucidate the initial steps.

Dissecting the initiation events that ultimately result i inum drug–induced apoptosis has proven difficult. One investigation that has produced some insight into this p has been the discovery of platinum-DNA damage recog proteins. The idea that a specific protein or protein co can bind to a platinum-DNA adduct and transmit a cell signal has intrigued researchers. Furthermore, mutati down-regulation of such a protein could result in or lead development of platinum drug resistance. Efforts to i such molecules have resulted in the discovery of several dates. The first of these were the high-mobility group pr HMG1 and HMG2.[48–50] These proteins are capable of b DNA as well as recognizing bent DNA structures, such produced by cisplatin. Interestingly, HMG1 has an affi adducts formed by cisplatin but not by the inactive tran isomer. The HMG domain, which consists of a highly ba amino acid motif, has been found in other proteins, r which are involved in gene expression.[51] Although a fun role for these proteins in platinum sensitivity and res has yet to be conclusively demonstrated, a number of i have emerged. It has been suggested that HMG doma teins are responsible for communicating the presence damage to either the repair machinery or to programm death pathways. Alternatively, the presence of platinu adducts could sequester HMG domain proteins and th vent their normal function or even shield DNA adduc being properly recognized by other cellular proteins. A tive role for this class of molecules awaits further study.

A number of other platinum-DNA damage recogniti teins have been identified, including histone H1, RNA p ase I transcription upstream binding factor (hUBF), th binding protein (TBP), and proteins involved in m repair (MMR). The latter have received significant because the recognition of platinum-DNA adducts by th complex has been implicated in cisplatin sensitivity.[52] have shown that the MSH2 and MLH1 proteins partic the recognition of DNA adducts formed by cisplatin.

presence of a platinum lesion may result in the continuous futile cycle of repair synthesis on the DNA strand opposite the lesion. This could result in the accumulation of DNA strand breaks and ultimately lead to cell death. Interestingly, oxaliplatin adducts are not well recognized by the MMR protein complex, which could account for differences in the cytotoxicity profiles observed between these two platinum complexes.

Although the specific proteins involved in platinum-DNA adduct recognition remain undefined, a number of signaling events have been shown to occur after treatment of a cell with cisplatin.[55] For example, the ATM- and Rad3-related protein (ATR), which is involved in cell-cycle checkpoint activation, is activated by cisplatin. This kinase, in turn, phosphorylates and activates several downstream effectors that regulate cell cycle, DNA repair, cell survival, and apoptosis. These include p53, CHK2, and members of the mitogen-activated protein kinase (MAPK) pathway [extracellular signal-related kinase (ERK), c-Jun amino-terminal kinase (JNK), p38 kinase]. The pleiotropic nature of this stress response only grows, because each of these molecules subsequently controls the activity and expression of many more proteins. As a result of this complexity, it is not surprising that a lack of consistency exists in conclusions drawn by investigators as to the role of these pathways in cell survival and apoptosis. This is also due to the various experimental conditions used, including differences in cell type, treatment, selection of end points, and duration of the effect. As an example, the role of p53 activation in the fate of platinum-treated cells has been a subject of debate. It is well known that p53 function is required for the activation of proapoptotic proteins such as the Bcl-2 family member Bax. However, disruption of p53 function has not always led to an observed decrease in cisplatin sensitivity. Two studies have shown that disrupting p53 function sensitizes cells to cisplatin, rather than causing them to be resistant.[56,57] One explanation for the increased sensitivity in p53-deficient cells is that a concomitant reduction in the cell-cycle inhibitor $p21^{Waf1/Cip1}$ causes cells to progress through G_2 and M unregulated. A premature mitosis may then occur in the presence of DNA damage, which results in cell death.

From these studies, it is apparent that the inherent sensitivity of a cell to any drug is influenced by a variety of factors. With respect to DNA-damaging agents such as cisplatin, the magnitude and duration of an apoptotic signal may be either enhanced or suppressed by the activity of other cellular signaling pathways. Thus, a damage or DNA adduct threshold may exist that is unique to each tumor cell and reflects the overall balance of prosurvival and proapoptotic signals. As the field of signal transduction has grown, so has the number of candidate effectors and pathways that may influence platinum drug sensitivity. The list is large and includes cytokines, growth factors, kinases, phosphatases, second messengers, transcription factors, redox proteins, and extracellular matrix proteins. Some of these molecules attenuate sensitivity only to platinum drugs and DNA-damaging agents, whereas others influence cellular sensitivity to a variety of unrelated chemotherapeutic drugs.

Some insight into the role of signaling in platinum drug sensitivity has been provided in studies using activators or inhibitors of known signal transduction pathways. For example, treatment of various cell lines with tamoxifen, epidermal growth factor, interleukin-1α, tumor necrosis factor-α, bombesin, and rapamycin enhances cisplatin cytotoxicity.[58–62] Also, the expression of certain protooncogenes, including *Ha-Ras*, *v-abl*, and Her2/*neu*, has been shown in some instances to promote cell survival after cisplatin exposure.[63–66] As mentioned earlier, members of the ERK/MAPK family as well as their upstream activators have been implicated in these events. The JNK/stress-activated protein kinase (SAPK) and p38 kinase pathways have been shown to be activated by a variety of environmental stimuli and inflammatory cytokines.[67] JNK/SAPK and p38 phosphorylate and regulate the activity of the ATF2 and Elk-1 transcription factors. JNK/SAPK also phosphorylates c-Jun, a component of the AP-1 transcription factor complex, on serine residues 63 and 73. There is considerable evidence to suggest that these protein kinases are involved in transmitting a drug-induced cell death signal. For example, Zanke et al.[68] demonstrated that in mouse fibroblasts, the inhibition of JNK phosphorylation by the stable transfection of a dominant-negative complementary DNA encoding SEK1, the protein kinase responsible for activating JNK, resulted in reduced sensitivity to cisplatin. Sanchez-Perez et al.[69] observed a prolonged activation of JNK by cisplatin that was related to cell death. Modulating the activity of kinases upstream of JNK, including c-Abl, MKK3/MKK6, MEKK1, and ASK1, also influences cellular drug sensitivity.[70] For example, Chen et al.[71] demonstrated that ov expression of a dominant-negative ASK1, which inhibits activation of JNK, resulted in an inhibition of cisplatin-induced apoptosis. Clearly, activation of these pathways occurs after drug exposure in some cells, and it is important to understand the contribution of these intracellular signaling events to overall platinum drug sensitivity.

MECHANISMS OF RESISTANCE

The major limitation to the successful treatment of solid tumors with platinum-based chemotherapy is the emergence of drug-resistant tumor cells.[55,72] Platinum drug resistance may be intrinsic or acquired and may occur through multiple mechanisms (Fig. 15.5-2). These mechanisms may be classified into two major groups: (1) those that limit the formation of cytotoxic platinum-DNA adducts, and (2) those that prevent cell death from occurring after platinum-DNA adduct formation. The first group of mechanisms includes decreased drug accumulation and increased drug inactivation by cellular prote and nonprotein thiols. The second group of mechani includes increased platinum-DNA adduct repair and increased platinum-DNA damage tolerance. Despite progress in the identification of specific proteins that are involved in platinum drug resistance, their relevance to clinical resistance remains to be defined. This is an important area of investigation, because the understanding of the molecular basis of the drug-resistant phenotype will lead to the development of reversal strategies.

REDUCED ACCUMULATION

The majority of cell lines that have been selected for cisplatin resistance *in vitro* exhibit a decreased platinum accumulation phenotype, and it is generally believed that this is due to decreased drug uptake rather than enhanced drug efflux. Cisplatin and its analogues may accumulate within cells by passive diffusion or facilitated transport.[73] Cisplatin uptake has been shown to be nonsaturable, even up to its solubility limit, and

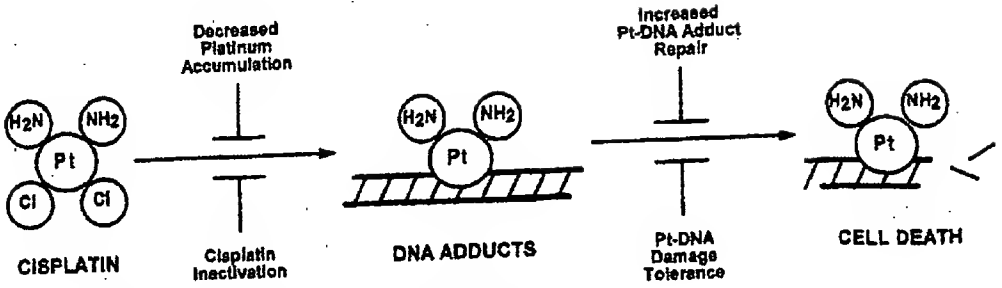

FIGURE 15.5-2. Cellular mechanisms of cisplatin resistance.

not inhibited by structural analogues. Carrier-mediated transport is supported by the observation that uptake is partially energy dependent, ouabain inhibitable, sodium dependent, and influenced by membrane potential and cyclic adenosine monophosphate levels. Although a specific human transporter has yet to be identified, progress has been made with respect to the identification of a copper transporter that can shuttle cisplatin into cells. In a study by Lin et al.[74] using a yeast model, the copper transporter CTR1 was shown to regulate the influx of cisplatin, carboplatin, oxaliplatin, and AMD473. Comparison of the wild-type and ctr-1 knockout strains revealed an eightfold reduction in cisplatin uptake after 1 hour. These ctr-1–deficient yeast cells were also twofold more resistant to cisplatin. These results increase the likelihood that analogous carrier-mediated transport pathways exist in human cells.

The prospect of an active efflux mechanism for platinum drugs has emerged after the discovery of a group of MRP-related transport proteins. MRP is a member of the ABC (adenosine triphosphate–binding cassette) family of transport proteins that participates in the extrusion of glutathione-coupled and unmodified anticancer drugs from cells.[75] Overexpression of MRP confers resistance to a variety of drugs, but not to cisplatin. For platinum complexes, the formation of a glutathione-platinum drug conjugate may be the rate-limiting step for producing an MRP substrate. The MRP homologue cMOAT (cannalicular multispecific organic anion transporter) shares 49% amino acid sequence identity and a similar substrate specificity with MRP. Taniguchi et al.[76] showed that cMOAT (MRP2) is overexpressed in some cisplatin-resistant human cancer cell lines exhibiting a decreased platinum accumulation phenotype. This group also demonstrated that transfection of an antisense cMOAT complementary DNA into HepG2 cells results in decreased cMOAT protein levels and a five-fold increase in cisplatin sensitivity.[77] Kool et al.[78] examined the expression of MRP, cMOAT, and three other MRP homologues (MRP3, MRP4, and MRP5) in a set of cell lines selected for cisplatin resistance *in vitro*. MRP1 and MRP4 messenger RNA levels were not increased in any of the cisplatin-resistant sublines. MRP3 and MRP5 were overexpressed in a few cell lines, but the messenger RNA levels were not associated with cisplatin resistance. In contrast, cMOAT was significantly overexpressed in some of the cisplatin-resistant cell lines. With respect to clinical relevance, an immunohistochemical analysis of the expression of P glycoprotein, MRP1, and MRP2 revealed that none of these transporters was associated with response to platinum-based chemotherapy in ovarian cancer.[79] Another class of proteins that is involved in the sequestration and efflux of platinum drugs is the copper-transporting P-type adenosine triphosphatases 7A and 7B (ATP7A and ATP7B). Transfection of epidermoid carcinoma cells with ATP7B led to a ninefold decrease in cisplatin sensitivity.[80] Howell has confirmed this and demonstrated that acquired cisplatin resistance is accompanied by increased expression of these pumps.[81,82] This group also found that increased expression of ATP7A is associated with poor survival in ovarian cancer patients treated with platinum-based regimens.[83]

INACTIVATION

The formation of conjugates between glutathione and platinum drugs may be an important step in their inactivation and elimination from the cell. For many years, investigators have attempted to make positive correlations between platinum drug sensitivity, glutathione levels, and the relative expression of the enzymes involved in glutathione metabolism. There have been many reports showing a strong association between platinum drug sensitivity and glutathione levels[84–87]; however, reducing intracellular glutathione levels with drugs such as buthionine sulfoximine has resulted in only low to moderate potentiation of cisplatin sensitivity.[88,89] Part of the reason for this may be due to the fact that the formation of glutathione-platinum conjugates is a slow process.[90] The formation of a glutathione-platinum complex, however, has been reported to occur in cultured cells, and glutathione has been shown to quench platinum-DNA monoadducts *in vitro*, preventing them from being converted to potentially cytotoxic cross-links. Another nonprotein thiol that has been implicated in platinum resistance is cysteinylglycine. This product is generated from glutathione catabolism by γ-glutamyltransferase. The affinity of cysteinylglycine for cisplatin is significantly higher than that of glutathione, and transfection studies have demonstrated that overexpression of γ-glutamyltransferase confers resistance to cisplatin.[94] One unresolved question is whether the initial reaction of platinum drugs with glutathione is catalyzed by glutathione S-transferases (GSTs). In support of this, a 10-fold increase in cisplatin resistance was reported in Chinese hamster cells transfected with the GSTπ isoenzyme.[95] In contrast, transfection of NIH3T3 cells with GSTπ resulted in hypersensitivity to cisplatin.[96] Studies attempting to associate GST activity with cisplatin sensitivity in cell lines and tumor biopsy specimens have failed to consistently show a positive correlation between GST expression or activity and cisplatin sensitivity.[86–88,97]

Inactivation of the platinum drugs may also occur through binding to the metallothionein (MT) proteins. The high

family of sulfhydryl-rich, low-molecular-weight proteins that participate in heavy metal binding and detoxification. *In vitro*, cisplatin binds stoichiometrically to MT, and up to ten molecules of cisplatin can be bound to one molecule of MT.[98] Kelley et al.[99] demonstrated that overexpression of the full-length MT-II_A in mouse C127 cells conferred a fourfold resistance to cisplatin. Furthermore, this group showed that embryonic fibroblasts isolated from MT-null mice were hypersensitive to cisplatin.[100] These studies clearly show that modulating MT levels can alter cisplatin sensitivity; however, the contribution of MT to clinical platinum drug resistance is unclear. In some cell lines, elevated MT levels have been shown to be associated with cisplatin resistance, whereas in others, they have not.[86,101] Studies with human tumors has shown that, in some instances, MT expression level is associated with response to chemotherapy. For example, a significant correlation between MT overexpression, and response and survival was reported in urothelial transitional cell carcinoma patients.[102] Overexpression of MT has also been observed in bladder tumors from patients for whom cisplatin chemotherapy failed.[103]

INCREASED DNA REPAIR

Once platinum-DNA adducts are formed, cells must either repair or tolerate the damage to survive. The capacity to rapidly and efficiently repair DNA damage clearly plays a role in determining a tumor cell's sensitivity to platinum drugs and other DNA-damaging agents. There is evidence to suggest that cell lines derived from tumors that are unusually sensitive to cisplatin, such as testicular nonseminomatous germ cell tumors, are deficient in their ability to repair platinum-DNA adducts.[104] Increased repair of platinum-DNA lesions in cisplatin-resistant cell lines as compared to their sensitive counterparts has been shown in several human cancer cell lines, including ovarian,[105,106] breast,[107] and glioma,[108] as well as murine leukemia cell lines.[109] Evidence for increased repair of cisplatin interstrand cross-links in specific gene and nongene regions in cisplatin-resistant cell lines has also been demonstrated. These studies have been done using a variety of *in vivo* methods, including unscheduled DNA synthesis, host cell reactivation of cisplatin-damaged plasmid DNA, atomic absorption spectrometry, quantitative polymerase chain reaction, and renaturing agarose gel electrophoresis.

The repair of platinum-DNA adducts occurs predominantly by nucleotide excision repair (NER); however, the molecular basis for the increased repair activity observed in cisplatin-resistant cells is unknown.[110] Because the rate-limiting step in this process is platinum adduct recognition and incision, increased expression of the proteins that control this step are likely to enhance NER activity. Using an *in vitro* assay, Ferry et al.[111] demonstrated that the addition of the ERCC1/XPF protein complex increased the platinum-DNA adduct excision activity of an ovarian cancer cell extract. There is also circumstantial evidence that implicates *ERCC1* expression in increased NER and cisplatin resistance. For example, expression levels of the *ERCC1* and *XPA* genes have been shown to be higher in malignant tissue from ovarian cancer patients resistant to platinum-based therapy than in tissue from those responsive to treatment.[112] *ERCC1* expression has also been shown to correlate with NER activity and cisplatin resistance in human ovarian cancer cells.[111] Increased levels of XPE, a putative DNA repair protein that recognizes many DNA lesions including platinum-DNA adducts, has been observed in tumor cell lines resistant to cisplatin.[113] It should be noted, however, that XPE is not a necessary component for the *in vitro* reconstitution of NER.[112,114] Increased expression of alpha-DNA polymerase and beta-DNA polymerase has been observed in cisplatin-resistant cell lines, and increased expression of these polymerases, as well as of DNA ligase, has been described in human tumors after cisplatin exposure *in vivo*.[108] The possible significance of these findings is unclear, because the primary polymerases involved in NER are thought to be delta-DNA polymerase or epsilon-DNA polymerase.[110] Although it is probably not involved in NER, beta-DNA polymerase may be involved in translesion DNA synthesis.[115]

Inhibiting DNA repair activity to enhance platinum drug sensitivity has been an active area of investigation. Selvakumaran et al.[116] showed that down-regulation of ERCC-1 using an antisense approach sensitized a platinum-resistant cell line to cisplatin both *in vitro* and *in vivo*. Pharmacologic agents have also been used, including nucleoside analogues such as gemcitabine, fludarabine, and cytarabine; the ribonucleotide reductase inhibitor hydroxyurea; and the inhibitor of alpha- and gamma-DNA polymerases aphidicolin. All of these agents interfere with the repair synthesis stage of various repair processes, including NER. It should be noted that these compounds are also likely to affect DNA replication, and as such should not be strictly characterized as repair inhibitors. The potentiation of cisplatin cytotoxicity by treatment with aphidicolin has been studied extensively in human ovarian cancer cell lines. Although some studies have demonstrated a clear synergism with this drug combination,[117,118] others have not.[119] In an *in vivo* mouse model of human ovarian cancer, the combined treatment of cisplatin and aphidicolin glycinate, a water-soluble form of the drug, was found to be significantly more effective than cisplatin alone.[120] The combination of cytarabine and hydroxyurea was found to demonstrate cytotoxic synergy with cisplatin in a human colon cancer cell line[121] and in rat mammary carcinoma cell lines.[122] Moreover, the modulatory effect of cytarabine and hydroxyurea on cisplatin was associated with an increase in DNA interstrand cross-links in both cellular systems. Similarly, the drugs gemcitabine[123] and fludarabine[124] have both been shown to synergize with cisplatin in causing cell death in *in vitro* systems, and both of these drugs have been shown to interfere with the removal of cisplatin-DNA adducts. The likelihood of a significant improvement in the therapeutic index of cisplatin in refractory patients by the coadministration of a repair inhibitor is limited, however, by the typically multifactorial nature of resistance in tumor cells. Combining an inhibitor of the repair process with other modulators of resistance may be a more viable avenue in treating patients with recurrent disease. Furthermore, a modest change in drug sensitivity may bring some refractory tumors into a range that is treatable with conventional chemotherapy.

INCREASED DNA DAMAGE TOLERANCE

After platinum-DNA adduct formation, the sensitivity of a cell depends on the efficiency with which DNA adducts are recognized and transmittal of a damage signal to the apoptotic machinery. Thus, any disruption, loss, or reduced activity of the components of this pathway(s) can result in a platinum-DNA

damage tolerance or multidrug resistance phenotype or both. Platinum-DNA damage tolerance has been observed in both cisplatin-resistant cells derived from chemotherapy-refractory patients and cells selected for primary cisplatin resistance *in vitro*. The contribution of this mechanism to resistance is significant, and it has been shown to correlate strongly with cisplatin resistance as well as resistance to other drugs in two ovarian cancer model systems.[106,125] Like other cisplatin resistance mechanisms, this phenotype may result from alterations in a variety of cellular pathways. Some of these individual mechanisms may confer resistance only to platinum drugs, whereas others may be responsible for multidrug resistance.

One component of DNA damage tolerance that has been observed in cisplatin-resistant cells involves the loss of function of the DNA MMR system. The main function of the MMR system is to scan newly synthesized DNA and remove mismatches that result from nucleotide incorporation errors made by the DNA polymerases. In addition to causing genomic instability, it has been reported that loss of MMR is associated with low-level cisplatin resistance and that the selection of cells in culture for resistance to this drug often yields cell lines that have lost a functional MMR system.[126] MMR deficiency may create an environment that promotes the accumulation of mutations in drug sensitivity genes. Another hypothesis is that the MMR system serves as a detector of platinum-DNA adducts. MSH2 alone, and in combination with MSH6, has been shown to bind to cisplatin 1,2-d(GpG)Pt intrastrand adducts with high efficiency.[54,127] In addition, MSH2- and MLH1-containing protein-DNA complexes have been observed when nuclear extracts of MMR-proficient cell lines are incubated with DNA preincubated with cisplatin, but not with oxaliplatin. These data suggest that MMR recognition of damage may trigger a programmed cell death pathway rendering cells with intact MMR more sensitive to DNA damage.[53] Another possibility is that the cytotoxicity involves repeated rounds of synthesis past the platinum-DNA lesions followed by recognition and subsequent removal of the newly synthesized strand by the MMR system. This futile cycling may generate DNA strand gaps and breaks that trigger programmed cell death.[128] Loss of MMR would thus increase the cell's ability to tolerate platinum-DNA lesions.

Another possible tolerance mechanism related to MMR is enhanced replicative bypass. This is defined as the ability of the replication complex to synthesize DNA past a platinum adduct.[115,129] Increased replicative bypass has been shown to occur in cisplatin-resistant human ovarian cancer cells.[129] These cells are also MMR deficient, and it was shown that in steady-state chain elongation assays, a 2.5- to 6.0-fold increase in replicative bypass of cisplatin adducts occurred. Oxaliplatin adducts are not recognized by the MMR complex, and no significant differences in bypass of oxaliplatin adducts in MMR-proficient and MMR-defective cells were observed. Beta-DNA polymerase, the most inaccurate of the DNA polymerases, may also function in this process.[115] The activity of this enzyme was found to be significantly increased in cells derived from a human malignant glioma resistant to cisplatin compared to its drug-sensitive counterpart.[108]

The tolerance mechanisms just described are related primarily to cisplatin resistance. Because the platinum-DNA damage tolerance phenotype is often associated with cross-resistance to other unrelated chemotherapeutic drugs,[125] the existence of a more general resistance mechanism must be considered. One possible explanation is that the platinum-DNA damage tolerance phenotype is the result of decreased expression or i vation of one or more components of the programme death pathway. As mentioned previously, a number of pro totic and antiapoptotic signaling pathways have been impli in cisplatin sensitivity. The possibility exists that cells conta defective or constitutively down-regulated stress signaling ways such as SAPK/JNK may exhibit resistance to cisplatin weight of the evidence favors a proapoptotic role for bot and p38 in tumor cells, whereas their role in normal c more equivocal.[68,69,130,131] Paradoxically, c-Jun, a target of may contribute to cisplatin resistance,[132,133] which speaks t importance of characterizing dimers in the MAPK pathwa composition of which may determine the balance of pro totic and antiapoptotic signaling.[130] Signaling for apopt oxaliplatin-treated cells appears qualitatively different fro in cisplatin-treated cells. Variation in the activity of the JN p38 pathways is not a determinant of cell death signali colon cancer cells, whereas resistance to oxaliplatin is enced very markedly by the activity of the NFκB pathway other cells the activity of ATF2, a substrate for JNK and p also a determinant of resistance.[135] The activity of these s ing pathways on mediators of apoptosis cannot easily be rated from effects on transcription of many of the mediat detoxification, DNA repair, and DNA damage toleranc cussed earlier in this chapter, and active research is in pr to test their role in the clinic.

Cell death may also be influenced by expression of me of the bcl-2 gene family. This group of proapoptotic and ant totic proteins regulates mitochondrial function and functio cell survival and cell death rheostat by forming homodime beterodimers with one another. The antiapoptotic bcl-2 ar X_L proteins are localized in the outer mitochondrial mem and may be involved in the formation of transmembrane nels. Overexpression of bcl-2 or bcl-X_L has been shown to p disruption of the mitochondrial transmembrane potential prolong cell survival in some cells after exposure to cisplat other anticancer drugs.[136,137] The activity of these prot negated, however, in the presence of high levels of the pr totic protein Bax, another bcl-2 family member. Therefo relative intracellular levels of these proteins may also confe tance to platinum drugs.

CLINICAL PHARMACOLOGY

PHARMACOKINETICS

The pharmacokinetic differences observed between pl drugs may be attributed to the structure of their leaving Platinum complexes containing leaving groups that are l ily displaced exhibit reduced plasma protein binding, plasma half-lives, and higher rates of renal clearance. Th tures are evident in the pharmacokinetic properties of ci carboplatin, and oxaliplatin, which are summarized i 15.5-1. Platinum drug pharmacokinetics have also been r elsewhere.[138,139]

Cisplatin

After intravenous infusion, cisplatin rapidly diffuses int and is covalently bound to plasma protein. More than

TABLE 15.5-1. Comparative Pharmacokinetics of Platinum Analogues after Bolus or Short Intravenous Infusion

	Cisplatin	*Carboplatin*	*Oxaliplatin*
$T_{1/2}\alpha$ (min)			
Total platinum	14–49	12–98	26
Ultrafiltrate	9–30	8–87	21
$T_{1/2}\beta$ (h)			
Total platinum	0.7–4.6	1.3–1.7	—
Ultrafiltrate	0.7–0.8	1.7–5.9	—
$T_{1/2}\gamma$ (h)			
Total platinum	24–127	8.2–40.0	38–47
Ultrafiltrate	—	—	24–27
Protein binding	>90%	24–50%	85%
Urinary excretion	23–50%	54–82%	>50%

$T_{1/2}\alpha$, half-life of first phase; $T_{1/2}\beta$, half-life of second phase; $T_{1/2}\gamma$, half-life of terminal phase.
(Data adapted from refs. 10 and 130–139.)

platinum is bound to plasma protein at 4 hours after infusion.[140] The disappearance of ultrafiltrable platinum is rapid and occurs in a biphasic fashion. Half-lives of 10 to 30 minutes and 0.7 to 0.8 hours have been reported for the initial and terminal phases, respectively.[141,142] Cisplatin excretion is dependent on renal function, which accounts for the majority of its elimination. The percentage of platinum excreted in the urine has been reported to be between 23% and 40% at 24 hours after infusion.[143,144] Only a small percentage of the total platinum is excreted in the bile.[145]

Carboplatin

The differences in pharmacokinetics observed between cisplatin and carboplatin depend primarily on the slower rate of conversion of carboplatin to a reactive species. Thus, the stability of carboplatin results in a low incidence of nephrotoxicity. Carboplatin diffuses rapidly into tissues after infusion; however, it is considerably more stable in plasma. Only 24% of a dose was bound to plasma protein at 4 hours after infusion.[146] The disappearance of platinum from plasma after short intravenous infusions of carboplatin has been reported to occur in a biphasic or triphasic manner. The initial half-lives for total platinum, which vary considerably among several studies, are listed in Table 15.5-1. The half-lives for total platinum range from 12 to 98 minutes during the first phase ($T_{1/2}\alpha$) and from 1.3 to 1.7 hours during the second phase ($T_{1/2}\beta$). Half-lives reported for the terminal phase range from 8.2 to 40 hours. The disappearance of ultrafiltrable platinum is biphasic with $T_{1/2}\alpha$ and $T_{1/2}\beta$ values ranging from 7.6 to 87 minutes and 1.7 to 5.9 hours, respectively. Carboplatin is excreted predominantly by the kidneys, and cumulative urinary excretion of platinum is 54% to 82%, most as unmodified carboplatin. The renal clearance of carboplatin is closely correlated with the glomerular filtration rate (GFR).[147] This observation enabled Calvert et al.[10] to design a carboplatin dosing formula based on the individual patient's GFR.

Oxaliplatin

After oxaliplatin infusion, platinum accumulates into three compartments: plasma bound platinum, ultrafiltrable platinum, and platinum associated with erythrocytes. When specific and sensitive mass spectrometric techniques are used, oxaliplatin itself is undetectable in plasma, even at end infusion.[148] The active forms of the drug have not been extensively characterized. Approximately 85% of the total platinum is bound to plasma protein at 2 to 5 hours after infusion.[149] Plasma elimination of total platinum and ultrafiltrates is biphasic. The half-lives for the initial and terminal phases are 26 minutes and 38.7 hours, respectively, for total platinum and 21 minutes and 24.2 hours, respectively, for ultrafiltrable platinum (see Table 15.5-1).[140] Thus, as with carboplatin, substantial differences between total and free platinum kinetics are not observed. As with cisplatin, a prolonged retention of oxaliplatin is observed in red blood cells. However, unlike cisplatin, oxaliplatin does not accumulate to any significant level after multiple courses of treatment.[149] This may explain why neurotoxicity associated with oxaliplatin is reversible. Oxaliplatin is eliminated predominantly by the kidneys, with more than 50% of the platinum being excreted in the urine at 48 hours.

PHARMACODYNAMICS

Pharmacodynamics relates pharmacokinetic indices of dr exposure to biologic measures of drug effect, usually toxicity to normal tissues or tumor cell kill. Two issues to be addressed in such studies are whether the effectiveness of the drug can be enhanced and whether the toxicity can be attenuated by knowledge of the platinum pharmacokinetics in an individual. These questions are appropriate to the use of cytotoxic agents with relatively narrow therapeutic indices. Toxicity to normal tissues can be quantitated as a continuous variable when the drug causes myelosuppression. Thus, the early studies of carboplatin demonstrated a close relationship of changes in platelet counts to the area under the concentration-time curve (AUC) in the individual. The AUC was itself closely related to renal function, which was determined as creatinine clearance. Based on these observations, Egorin et al.[11] and Calvert et al.[10] derived formulas based on creatinine clearance to predict either the percentage change in platelet count or a target AUC. More recently, Chatelut and colleagues[150] have derived a formula that relies on serum creatinine levels as well as morphometric determinants of renal function. Application of pharmacodynamically guided dosing algorithms for carboplatin has been widely adopted as a means of avoiding overdosage (by produc acceptable nadir platelet counts) and of maximizing dose intensity in the individual. There is good evidence that this approach can decrease the risk of unacceptable toxicity. Accordingly, a dosing strategy based on renal function is recommended for the use of carboplatin.

A key question is whether maximizing carboplatin exposure in an individual can measurably increase the probability of tumor regression or survival. In an analysis by Jodrell et al.,[151] carboplatin AUC was a predictor of response, thrombocytopenia, and leukopenia. The likelihood of a tumor response increased with increasing AUC up to a level of 5 to 7 mg × h/mL, after which a plateau was reached. Similar results were obtained with carboplatin in combination with cyclophosphamide, and neither response rate nor survival was determined by the carboplatin AUC in a cohort of ovarian cancer patients.[152]

The relationship of pharmacokinetics to response may also be explored by investigating the cellular pharmacology of

these agents.[155] As discussed in DNA Adduct Formation, earlier in this chapter, platinum compounds form various types of DNA adducts. The formation and repair of these adducts in human cells are not easily measured. One approach is to measure specific DNA adducts (using antibody-based assays), whereas another is to measure total platinum bound to DNA. The formation and repair of platinum-DNA adducts has been studied in white blood cells obtained from various groups of patients. Schellens and colleagues[154,155] have reevaluated the pharmacokinetic and pharmacodynamic interactions of cisplatin administered as a single agent. In a series of patients with head and neck cancer, they found that cisplatin exposure (measured as the AUC) closely correlated with both the peak DNA adduct content in leukocytes and the area under the DNA-adduct-time curve. These measures were important predictors of response, both individually and in logistic regression analysis.

PHARMACOGENOMICS

Variability in pharmacokinetics and pharmacodynamics of cytotoxic drugs is an important determinant of therapeutic index. This interindividual variation may be attributed in part to genetic differences among patients. For platinum drugs, genetic differences underlying pharmacokinetic variation have not been described. Several groups are actively investigating the basis of pharmacodynamic variation, and the initial work has focused on proteins that are involved in some of the mechanisms described in Mechanism of Action, earlier in this chapter. Detoxification pathways and DNA repair have been studied in several clinical trials. Single nucleotide polymorphisms in genes related to glutathione metabolism and in ERCC genes have been identified in small studies,[156] but larger scale studies have not confirmed early findings. These early studies have much promise, however, both to identify patients with greater or lesser toxicity from standard dosages and to determine subgroups of patients with differing probabilities of response.

FORMULATION AND ADMINISTRATION

CISPLATIN (PLATINOL)

Cisplatin is administered in a chloride-containing solution intravenously over 0.5 to 2.0 hours. To minimize the risk of nephrotoxicity, patients are prehydrated with at least 500 mL of salt-containing fluid. Immediately before cisplatin administration, mannitol (12.5 to 25.0 g) is given parenterally to maximize urine flow. A diuretic such as furosemide may be used also, along with parenteral antiemetics. These currently include dexamethasone together with a 5-hydroxytryptamine (5-HT_3) antagonist. A minimum of 1 L of posthydration fluid is usually given.[157] The intensity of hydration varies somewhat with the dose of cisplatin. High-dose cisplatin (up to 200 mg/m^2/course) may be administered in a formulation containing 3% sodium chloride, but this method is no longer widely used. Cisplatin may also be administered regionally to increase local drug exposure and diminish side effects. Its intraperitoneal use was defined by Ozols et al.[158] and by Howell and colleagues.[159] Measured drug exposure in the peritoneal cavity is some 50-fold higher compared to levels achieved with intravenous administration.[159] At dosages in ovarian cancer patients with low-volume disea domized intergroup trial suggested that intraperitoneal tration is superior to intravenous cisplatin in combinat intravenous cyclophosphamide.[160] The development of c tions of carboplatin and paclitaxel has, however, superse technique in treatment of ovarian cancer, and the intrape route is now infrequently used. Regional uses also inclu arterial delivery (as for hepatic tumors, melanoma, and toma), but none has been adopted as a standard me treatment. There is growing interest in chemoemboliza the treatment of tumors confined to the liver, and cispl component of many popular regimens.[161]

CARBOPLATIN (PARAPLATIN)

Cisplatin treatment over 3 to 6 hours is burdensome for resources and tiring for cancer patients. Previously g in-hospital treatment, it is now usually administered in patient setting. The exigencies of the modern health ca ronment have contributed to the expanding use of carb as an alternative to cisplatin except in circumstances in cisplatin is clearly the superior agent. Carboplatin is subst easier to administer. Extensive hydration is not required b of the lack of nephrotoxicity at standard dosages.[162] Carb is reconstituted in chloride-free solutions (unlike ci because chloride can displace the leaving groups) and a tered over 30 minutes as a rapid intravenous infusion. platin has been incorporated in high-dose chemo regimens at dosages over threefold higher than those standard regimens.[163] In some regimens, continuous i has been substituted for a rapid intravenous infusion; hov is doubtful that there is an advantage to this approach. platin dosages up to 20 mg × min/mL may be safely a tered in 200 mL of dextrose 5% in water over 2 hours.[164]

OXALIPLATIN (ELOXATIN)

Oxaliplatin is also uncomplicated in its clinical adm tion. For bolus infusion, the required dose is administ 500 mL of chloride-free diluent over a period of 2 h studies of colorectal cancer, oxaliplatin has been a tered as a 5-day continuous infusion, during which the rate has been modified to observe principles of chro macologic administration.[165] Oxaliplatin is more fre given as a single dose every 2 weeks (85 mg/m^2) or weeks (130 mg/m^2), alone or with other active ager common to pretreat patients with active antiemetics, s 5-HT_3 antagonist, but the nausea is not as severe as platin. No prehydration is required. The predominat ity of oxaliplatin is neurotoxicity: The developmen oropharyngeal dysesthesia, often precipitated by exp cold, requires prolongation of the duration of admin to 6 hours. On occasion, the occurrence of hyperse requires slowing of the infusion also.

TOXICITY

A substantial body of literature documents the side e platinum compounds. The nephrotoxicity of cisplati

TABLE 15.5-2. Toxicity Profiles of Platinum Analogues in Clinical Use

Toxicity	*Cisplatin*	*Carboplatin*	*Oxaliplatin*
Myelosuppression		X	
Nephrotoxicity	X		
Neurotoxicity	X		X
Ototoxicity	X		
Nausea and vomiting	X	X	X

led to its abandonment, until Cvitkovic and colleagues introduced aggressive hydration, which prevented the development of acute renal failure.[3,4] As noted in History, earlier in this chapter, the toxicity of cisplatin was a driving force both in the search for less toxic analogues and for more effective treatments for its side effects, especially nausea and vomiting. The toxicities associated with cisplatin, carboplatin, and oxaliplatin are described in detail in the following sections and summarized in Table 15.5-2.

CISPLATIN

The side effects associated with cisplatin (at single doses of more than 50 mg/m^2) include nausea and vomiting, nephrotoxicity, ototoxicity, neuropathy, and myelosuppression. Rare effects include visual impairment, seizures, arrhythmias, acute ischemic vascular events, glucose intolerance, and pancreatitis.[157] The nausea and vomiting stimulated a search for new antiemetics. These effects are currently best managed with 5-HT_3 antagonists, usually given with a glucocorticoid, although other combinations of agents are still widely used. In the weeks after treatment, continuous antiemetic therapy may be required. Nephrotoxicity is ameliorated but not completely prevented by hydration. The renal damage to both glomeruli and tubules is cumulative, and after cisplatin treatment, serum creatinine level is no longer a reliable guide to GFR. An acute elevation of serum creatinine level may follow a cisplatin dose, but this index returns to normal with time. Tubule damage may be reflected in a salt-losing syndrome that also resolves with time.

Ototoxicity is a cumulative and irreversible side effect of cisplatin treatment that results from damage to the inner ear. Therefore, audiograms are recommended every two to three cycles.[157] The initial audiographic manifestation is loss of high-frequency acuity (4000 to 8000 Hz). When acuity is affected in the range of speech, cisplatin should be discontinued under most circumstances and carboplatin substituted where appropriate. Peripheral neuropathy is also cumulative, although less common than with agents such as vinca alkaloids. This neuropathy is usually reversible, although recovery is often slow. A number of agents with the potential for protection from neuropathy have been developed, but none is yet used widely.[166]

CARBOPLATIN

Myelosuppression, which is not usually severe with cisplatin, is the dose-limiting toxicity of carboplatin.[152] The drug is most toxic to the platelet precursors, but neutropenia and anemia are frequently observed. The lowest platelet counts after a single dose of carboplatin are observed 17 to 21 days later, and recovery usually occurs by day 28. The effect is dose dependent, but individuals vary widely in their susceptibility. As shown by Egorin et al.[11] and Calvert et al.,[10] the severity of platelet toxicity is best accounted for by a measure of the drug exposure in an individual, the AUC. Both groups derived pharmacologically based formulas to predict toxicity and guide carboplatin dosing. That of Calvert and colleagues targets a particular exposure to carboplatin:

$$\text{Dose (mg)} = \text{target AUC (mg} \cdot \text{min/mL)} \times (\text{GFR mL/min} + 25)$$

This formula has been widely used to individualize carboplatin dosing and permits targeting at an acceptable level of toxicity. Patients who are elderly or have a poor performance status, or have a history of extensive pretreatment have a higher risk of toxicity even when dosage is calculated with these methods,[10,11] but the safety of drug administration has been enhanced. In the combination of carboplatin and paclitaxel, AUC-based dosing has helped to maximize the dose intensity of carboplatin.[167] Dosages some 30% higher than those using a dosing strategy based solely on body surface area may safely be used. A determination of whether this approach to dosing improves outcome will require a randomized trial.

The other toxicities of carboplatin are generally milder and better tolerated than those of cisplatin. Nausea and vomiting, although frequent, are less severe, shorter in duration, and more easily controlled with standard antiemetics [i.e., prochlorperazine (Compazine)], dexamethasone, lorazepam) than that after cisplatin treatment. Renal impairment is infrequent, although alopecia is common, especially with the paclitaxel-containing combinations. Neurotoxicity is also less common than with cisplatin, although it is observed more frequently with the increasing use of high-dose regimens. Ototoxicity is also less common.

OXALIPLATIN

The dose-limiting toxicity of oxaliplatin is sensory neuropathy, a characteristic of all DACH-containing platinum derivatives. The severity of the toxicity is dramatically less than that observed with another DACH-containing analogue, ormaplatin. This side effect takes two forms. First, a tingling of the extremities, which may also involve the perioral region, that occurs early and usually resolves within a few days. With repeated c ing, symptoms may last longer between cycles, but do not appear to be of long duration or cumulative. Laryngopharyngeal spasm and cold dysesthesias have also been reported but are not associated with significant respiratory symptoms and can be prevented by prolonging the duration of infusion. A second neuropathy, more typical of that seen with cisplatin, affects the extremities and increases with repeated doses. Definitive physiologic characterization of oxaliplatin-induced neuropathy has proven difficult in large studies. Electromyograms performed in six patients treated by Extra et al.[18] revealed an axonal sensory neuropathy, but nerve conduction velocities were unchanged. Specimens from peripheral nerve biopsies performed in this study showed decreased myelinization and replacement with collagen pockets. The neurologic effects of oxaliplatin appear to be cumulative in that they become more pronounced and of greater duration with successive cycles; however, unlike those of cisplatin, they are revers-

ible with drug cessation. In a review of 682 patient experiences, Brienza et al.[168] reported that 82% of patients who experienced grade 2 neurotoxicity or higher had their symptoms regress within 4 to 6 months. In a larger adjuvant trial, de Gramont et al.[169] reported that 12% of patients had grade 3 toxicity at the end of a 6-month treatment period and that the majority of these patients had relief, but not always complete resolution of the symptoms, by 1 year later. The persistence of the neurotoxicity has led to approaches to ameliorate it, including the use of protective agents (calcium and magnesium salts intravenously before and after each infusion)[149] or a more intensive schedule initially, followed by interruption of the oxaliplatin component of the chemotherapy for a few cycles.[169] Ototoxicity is not observed with oxaliplatin. Nausea and vomiting do occur and generally respond to 5-HT_3 antagonists. Myelosuppression is uncommon and is not severe with oxaliplatin as a single agent, but it is a feature of combinations including this drug. Oxaliplatin therapy is not associated with nephrotoxicity.

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SECTION 6

Antimetabolites

METHOTREXATE

Aminopterin was the first antimetabolite to demonstrate clinical activity in the treatment of patients with malignancy. This antifolate analogue was used to induce remissions in children with acute leukemia in the 1940s. Aminopterin was subsequently replaced by methotrexate (MTX), the 4-amino, 10-methyl analogue of folic acid. MTX remains the most widely used antifolate in cancer chemotherapy, with documented activity against a wide range of human malignancies, including many solid tumors and hematologic malignancies. Antifolates have also been used to treat a host of nonmalignant disorders, including psoriasis, rheumatoid arthritis, graft-versus-host disease, bacterial and plasmodial infections, and parasitic infections associated with acquired immunodeficiency syndrome. This class of agents represents the best-characterized and most versatile of all chemotherapeutic drugs in current clinical use.

MECHANISM OF ACTION

MTX is a tight-binding inhibitor of dihydrofolate reductase (DHFR), a critical enzyme in folate metabolism (Fig. 15.6-1).